Sporadic Fatal Insomnia in Europe

Sporadic Fatal Insomnia in Europe: Phenotypic features and diagnostic challenges.

Running head: sFI in Europe

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ABSTRACT

Objective: Comprehensively describe the phenotypic spectrum of sporadic Fatal Insomnia (sFI) to facilitate diagnosis and management of this rare and peculiar prion disorder.

Methods: A survey among major prion disease reference Centres in Europe identified 13 patients diagnosed with sFI in the past 20 years. We undertook a detailed analysis of clinical and histopathological features, and the results of diagnostic investigations.

Results: Mean age at onset was 43 years and mean disease duration 30 months. Early clinical findings included psychiatric, sleep and oculomotor disturbances, followed by cognitive decline and postural instability. In all tested patients, video-polysomnography demonstrated a severe reduction of total sleep time and/or a disorganized sleep. CSF levels of proteins 14-3-3 and t-tau were unrevealing, the concentration of neurofilament light protein (NfL) was more consistently increased, and the real-time quaking-induced conversion assay (RT-QuIC) revealed a positive prion seeding activity in 60% of cases. EEG and MRI showed non-specific findings, whereas FDG-PET demonstrated a profound bilateral thalamic hypometabolism in 71% of cases. Molecular analyses revealed PrP^{Sc} type 2 and methionine homozygosity at *PRNP* codon 129 in all cases.

Interpretation: sFI is a disease of young or middle-aged adults, which is difficult to reconcile with the hypothesis of a spontaneous etiology related to stochastic, age-related, PrP misfolding. The combination of psychiatric and/or sleep-related symptoms with oculomotor abnormalities represent early peculiar clinical feature of sFI to be valued in the differential diagnosis. Video-polysomnography, FDG-PET, and especially CSF prion RT-QuIC and NfL constitute the most promising supportive diagnostic tests *in vivo*.

INTRODUCTION

Human prion diseases are rapidly progressive neurodegenerative disorders pathogenically related to structural changes of the cellular prion protein (PrP^{c}). In prion disease, PrP^{c} converts to a beta-sheet rich, partially protease-resistant form, termed PrP^{Sc}, which accumulates in the brain and other tissues.

Prion diseases uniquely occur in inherited, sporadic or acquired forms and show a broad clinical and pathological heterogeneity. Four major phenotypic entities are recognized in humans, namely, Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome (GSS), Fatal Insomnia (FI) and variably protease-sensitive prionopathy (VPSPr)[1-3]. The phenotypic spectrum of CJD, by far the most common form, largely correlates at molecular level with two major PrP^{Sc} types with distinct physicochemical properties, designated as types 1 and 2. Moreover, the genotype at the polymorphic methionine/valine (M/V) codon 129 of prion protein gene (*PRNP*) also plays a role. Accordingly, the current classification of sporadic CJD (sCJD) recognizes six major subtypes largely corresponding to all possible combinations of the two molecular variables (e.g. MM1, MM2, VV1, VV2, etc.)[4,5]. The most significant exception is the MM2 group, which includes two subtypes designated as MM2-cortical (MM2C) and MM2-thalamic (MM2T) based on distinctive pathological features[4].

Given its striking clinical and neuropathologic similarities with Fatal Familial Insomnia (FFI), a genetic prion disease linked to a point mutation at codon 178 (D178N) in the *PRNP* coupled with methionine at codon 129, the MM2T subtype is also known as sporadic Fatal Insomnia (sFI)[6,7]. Transmission studies using susceptible transgenic mice have consistently demonstrated that the same prion strain is associated with both sFI and FFI[8,9].

In contrast to what has been the rule for the most common neurodegenerative disorders, sFI is rarer than its genetic counterpart[10]. Indeed, while the recognized patients with FFI are numerous and belong to more than 50 families worldwide, only about 30 cases of CJD MM2T and a few cases with mixed MM2T and MM2C features (MM2T+C) have been recorded to date[6,8,9,11-23].

Moreover, while the first description of FFI dates back to 1986 and its recognition as a genetic prion disease to 1992, the characterization of sFI as a distinct sporadic prion disease subtype was only achieved in 1999[6,8]. It is noteworthy, however, that a few cases previously reported under the terms thalamic dementia, thalamic degeneration or thalamic form of CJD, either sporadic or familial, likely belonged to the same disease entity[1,24-32].

Here we describe in detail the clinical findings, results of diagnostic investigations and neuropathological features of the largest series of sFI patients reported to date, representing the result of 20 years of prion disease surveillance in Europe.

SUBJECTS AND METHODS

Patient selection.

After inquiry to the majority of European CJD reference Centers, we identified 13 cases of sCJD MM2T or sFI, which were diagnosed in Italy (n=7, patients #1 to 6 and patient #13), the Netherlands (n=1, patient #7), Spain (n=1, patient #8), United Kingdom (n=2, patients #9 and 10), Germany (n=2, patients #11 and 12), over the past 20 years. Reference laboratories for CJD in France, Austria, Poland, Belgium and Denmark did not report any case. A single sCJD MM2T+C, recently reported in Spain[23] was not included in the present series.

The diagnosis of sCJD MM2T was formulated post-mortem, according to neuropathologic consensus criteria[33] in 12 cases. One additional patient (case #13) was diagnosed as probable sCJD MM2T-type based on typical clinical features, polysomnographic and fluorodeoxyglucose-positron emission tomography (FDG-PET) findings, and the new diagnostic criteria for sCJD (i.e. progressive neurological syndrome and positive CSF RT-QuIC assay; http://www.cjd.ed.ac.uk/sites/default/files/criteria_0.pdf).

Clinical data and diagnostic investigations.

For each patient, we collected and reviewed data regarding clinical history, neurological examination and the specific evaluation of cognitive status, which in the majority of cases, included neuropsychological batteries.

We also reviewed copies and collected data concerning electroencephalographic recordings (EEG) (n=13), video-polysomnography (n=4) or long-term video-EEG (n=1), autonomic function tests (n=6), neurohormonal assays (n=3), brain magnetic resonance imaging (MRI) (n=13), FDG-PET (n=7) and Tc^{99m} HMPAO or ECD cerebral blood flow single photon emission computed tomography (CBF-SPECT) (n=3). EEG and MRI findings were classified according to established criteria[34,35]. The most abnormal result on record considered in cases with serial EEG or neuroimaging examinations.

In the absence of a standardized protocol, MRI scans variably included T1/T2-weighted scans (n=13), fluid attenuated inversion recovery (FLAIR) sequences (n=11), diffusion weighted imaging (DWI) (n=11) and/or H^1-spectroscopy (n=3).

Video-polysomnographies, tests of autonomic function, neurohormonal assays, brain PET and CBF-SPECT were performed at the notifying hospitals according to standard procedures.

CSF biomarker analyses included proteins 14-3-3 (n=12), total (t)-tau (n=10), neurofilament light chain (NfL, n=3), and prion seeding activity (n=5). The 14-3-3 protein was measured semi-quantitatively by immunoblotting, whereas total-tau and NfL proteins were quantitatively analyzed using commercially available kits based on a sandwich ELISA method, as described[36,37]. Prion seeding activity was assessed by RT-QuIC as previously described[38].

PRNP (Ref. Seq. NM_0003111) open reading frame was analyzed as previously described in 12 cases[39].

Post-mortem studies.

Each brain was studied by routine histopathology, PrP immunohistochemistry and PrP^{Sc} typing by western blotting at the referring laboratories according to established procedures. However, to facilitate the comparison among cases and harmonize the evaluation, we re-processed (H&E staining and PrP immunohistochemistry) unstained sections from several brain regions, including, the cerebral cortices of each lobe, striatum, thalamus, hypothalamus (N=7), hippocampus, midbrain, medulla (n=8) and cerebellum in 10 out of the 12 cases. In the remaining two[9], one of us (PP) reviewed the original stained slides provided by the reference laboratory. For PrP immunohistochemistry, we immunolabeled paraffin sections from formalin-fixed, formic acid treated tissue blocks using the monoclonal antibody 3F4, according to a published protocol[40].

We also carried out a semiquantitative evaluation of spongiform change by comparing haematoxylin and eosin (H&E) stained sections obtained from eight brain regions. We scored the spongiform change as follows: absent: -, mild: +, moderate: ++, severe: +++, and status spongiosus: SS.

Furthermore, we determined the type and relative amount of PrP^{Sc} on 10% brain homogenates, after treatment with proteinase K, according to established consensus protocols[41].

Patient #1, 5 and 6 were briefly described in previous studies[9,16].

We conducted the study according to the revised Declaration of Helsinki and Good Clinical Practice guidelines. The protocol for this study received prior approval by the IRCCS-ISNB Institutional Review Board. Informed consent was obtained from each subject or next of kin.

RESULTS

Demographic and clinical features.

The mean age at symptom onset was 43 years (range 24-80) and the mean duration of clinical disease 30 months (range 7-96) (Table 1).

Family history was unremarkable except for patient #1 who, despite the non-mutated carrier status, belonged to a large kindred with several FFI affected members, as previously described[16]. Prominent clinical manifestations at onset included psychiatric, sleep, and oculomotor/visual disturbances (Fig 1). Among the former, the most frequent symptoms included mood alterations (n=11 patients, 84.6%), behavioural and personality changes (n=7, 53.8%), delusions (n=7, 53.8%), and hallucinations (n=2, 15.4%). Eleven patients (84.6%) complained of sleep disturbances, including drug-resistant insomnia (n=10, 76.9%), simple or complex movements during sleep (n=7, 53.8%), diurnal drowsiness (n=5, 38.5%) and sleep vocalizations (n=3, 23.1%). Moreover, oculomotor dysfunction and/or gaze palsy affected 11 (84.6%) patients causing diplopia in seven (53.8%).

In the full-blown disease, all subjects developed progressive cognitive impairment and postural instability associated with retro/lateropulsion and gait disturbances, while only some suffered additional motor abnormalities. Specifically, based on the results of consecutive neurological evaluations and of administered neuropsychological batteries the most affected cognitive domains included memory (12/12, 100%), temporal and/or spatial orientation (9/12, 75.0%), language (9/12, 75.0%) and executive functions (9/12, 75.0%) and attention (7/12, 58.3%). Moreover, 5/12 (41.7%) of patients had agnosia, 4/12 (33.3%) apraxia and 4/12 (33.3%) visuospatial deficits.

Typical motor features were dysarthria (n=12, 92.3%), cerebellar signs (n=10, 76.9%), pyramidal signs (n=10, 76.9%), myoclonus (n=10, 76.9%), diurnal-nocturnal motor hyperactivity (n=8, 61.5%) and extrapyramidal signs (n=13, 100%). The latter comprised a hypokinetic-rigid syndrome in 12 patients (92.3%), hyperkinetic involuntary movements (mainly chorea) in four (30.8%), and limbs dystonia in four (30.8%).

Finally, five (38.5%) patients showed signs of autonomic hyperactivity, such as systemic hypertension (often drug-resistant) (n=3, 23.1%), mild pyrexia (n=3, 23.1%), hyperhidrosis (n=3, 23.1%), neurogenic urinary alterations (n=3, 23.1%) tachycardia (n=2, 15.3%), and constipation (n=2, 15.3%).

In addition, significant weight loss was reported in five cases (38.4%) (Table 1). Seven patients (53.8%) evolved to akinetic mutism, although this may not reliably represent the real frequency of this condition given that some medical reports contained only scanty information about the terminal disease stage (Fig 1).

Results of diagnostic investigations

CSF biomarkers (Table 2)

The 14-3-3 assay was negative in all examined cases, t-tau was within normal levels in seven cases, even when repeated at different clinical stages (patients #5, 12 and 13), moderately increased in one (patient 1), and increased at a level compatible with a diagnosis of probable CJD in only one case (patient #2). Finally, RT-QuIC was positive in three out of five examined cases. In all tested patients, NfL levels were significantly higher than in healthy controls and other dementia groups as previously described[37].

Genetic analysis

All cases were methionine homozygous at codon 129 (129M/M). The presence of the D178N mutation linked to FFI or any other *PRNP* mutation was excluded in 12 out of 13 patients by direct sequencing. In the only case with the non-sequenced *PRNP* ORF, PrP^{Sc} typing by western blotting showed that the intermediate monoglycosylated PrP^{Sc} glycoform was the most abundant, as in all other sporadic MM2T cases. Given that PrP^{Sc} in FFI invariably presents a predominant upper (diglycosylated) glycoform, this result indirectly excluded the presence of the D178N mutation also in this case[6].

EEG and video-polysomnography

EEG recordings were either normal (2/13, 15.4%) (cases #10 and 11) or showed a slow background activity characterized by dominant theta-delta rhythm (11/13, 84.6%). Periodic sharp-waves complexes (PSWC) were only seen in one out of 13 patients (7.7%) (case #2) in a late recording performed only a month before death.

Video-polysomnographic evaluation or long-term video-EEG monitoring (cases #1, 2, 3, 8 and 13) demonstrated a severe reduction of total sleep time and/or a completely disorganized sleep architecture and microstructure in tested patients. Specifically, in four out of five of them (80%) (cases #1, 2, 3 and 13), wakefulness appeared interchangeably alternated with a subwakefulness state (N1 sleep stage, light stupor) and, occasionally, with brief recurrent episodes of REM sleep while K-complexes, spindle and delta sleep, which are the hallmark of non-rapid eye movement sleep stages N2 and N3, were severely reduced or supressed. A reduction of total sleep time was also documented in the fifth case (20%) (patient #8), although detailed information about sleep microstructure were not available in this case. Hypnic disruption until complete loss of physiological sleep seem to occur progressively in sFI, as documented by serial polysomnographic recordings in patient #1 (Fig 2).

Moreover, episodes of oneiric stupor, a complex motor behaviour arising from subwakefulness, characterized by occurrence of stereotyped gestures mimicking daily life activities, were documented in two cases (40%) (suppl. Video). Finally, in one patient (20%) (patient #2), polysomnographic recording revealed a severe disruption of sleep architecture and microstructure even in absence of any subjective complain of sleep disturbance.

***Autonomic function and neurohormonal tests* (*Table 3*)**

Autonomic function and neurohormonal tests were performed in all five cases manifesting signs of autonomic overactivity (cases #1, 3, 6, 9 and 13) and in an additional patient (case #8) lacking them. The results showed a sympathetic hyperactivity in one patient (16.7%) (case #1), a drug-resistant systemic hypertension in another one (16.7%) (case #6), and a normal function in the other four (66.6%).

Neuroimaging

Single or consecutive MRI exams were unremarkable in five patients (38.5%) (cases #4, 8, 9, 11, 13). In all the others, brain MRI showed, mainly in the late disease stages, a mild to moderate atrophy of the

brain (8/13, 61.5%) (cases #1, 2, 3, 5, 6, 7, 10, 12), cerebellum (3/13, 23.1%) (cases #3, 5, 6) and/or the brainstem at the level of inferior olives and pons (1/13, 7.7%) (case #5). Cortical hyperintensities were observed only in one (9.1%) (case #1) out of eleven patients who underwent DWI and/or FLAIR sequences (Fig 3A). Finally, a nonspecific leukoencephalopathy was reported in one patient (9.1%) (case #6).

Brain spectroscopy MRI showed metabolic alterations compatible with thalamic and cortical neurodegeneration in one case (patient #1), while it was unremarkable in 2 other cases (patients #3 and 13).

FDG-PET studies revealed a pronounced bilateral thalamic hypometabolism and scattered hypometabolic cortical areas (Fig 3B and 3C) in five out of seven patients (71.4%) (cases #1, 3, 5, 6 and 13), while they were unremarkable in two (28.6%) (patients #7 and 8).

Furthermore, 99mTc-HMPAO or ECD-SPECT scans showed abnormal cortical perfusion, but no thalamic alterations in two out of 3 patients (66.7%) (cases #2 and 7) and was unremarkable in one case (33.3%) (case #8).

Neuropathological findings

All brains of 12 definite cases fulfilled the consensus criteria for the histopathological diagnosis of sCJD MM2T[33] (Table 4 and Fig. 4). Overall, the results confirmed the very distinctive traits that define the sCJD MM2T histotype, although they also showed a few novel features. Indeed, all cases demonstrated a moderate to severe neuronal loss and astrogliosis in the mediodorsal (Fig 4A and B) and anterior nuclei of the thalamus and in the inferior olivary nuclei (Fig 4C and D) but a relative sparing of striatum (Fig 4I) and hippocampus (CA and subiculum). Moreover, all showed a moderate Bergmann gliosis and torpedo formation in the cerebellum (Fig 4H) and most had a mild to moderate patchy spongiform change in the cerebral cortex (Fig 4E and F). As previously shown in FFI[42,43], disease duration significantly affected the spread and severity of histopathology. Accordingly, the two

cases with a moderate atrophy and most focally distributed changes had the shortest course, while those with the most severe thalamic degeneration, extending beyond the most affected nuclei listed above, had the longest course. Furthermore, while the two cases with the shortest duration of symptoms (7 and 8 months) only showed patchy, mild foci of spongiform change in the cerebral cortex with only mild neurodegenerative changes, the histopathological changes progressed to status spongiosus and severe atrophy in the two cases with the longest course (54 and 96 months) (Fig 4G). Moreover, the striatum was relatively unaffected in the cases with the shortest course, but florid spongiform change was observed in the case with the longest course, although with limited associated neuronal loss (Fig 4J). Finally, in a subgroup of cases with sufficient available material (7/12 patients, Table 4), the hypothalamus showed focal pathology characterized by mild to moderate astrogliosis, as previously described in FFI[42,43].

PrP immunohistochemistry revealed some new features, possibly related to disease duration. Indeed, while most cases only had a patchy PrP positivity of synaptic (not shown) or small granular type mostly in the superficial layer of the cerebral cortex (Fig 4K), one of the two patients with the longest course demonstrated sparse, small plaque-like deposits in the cerebral cortex (Fig 4L).

PrP^{Sc} typing by Western blot analyses confirmed the presence of type 2 in all definite cases (Fig 4M). As previously shown[6], PrP^{Sc} was characterized in all cases, and at variance with FFI, by a predominant monoglycosylated form and the lack of under-representation of the unglycosylated isoform.

DISCUSSION

We have described clinical findings, results of diagnostic investigations and neuropathological features of the largest series of sFI patients reported to date, reflecting the results of about 20 years of prion disease surveillance in Europe. The results expand the phenotypic spectrum of the disease and provide an update on the clinical impact of current available diagnostic investigations.

Age at onset (mean 43 years) in the present series was even younger than previously reported in sFI cases (mean 46 years) or in FFI patients carrying MM at codon 129 (mean 51 years) and significantly younger than in typical sCJD patients (mean 67 years)[6-9,11-23,44-46]. The result confirms that sFI is a disease of young or middle-aged adults rather than of the elderly, which is intriguing since this is difficult to reconcile with the current leading hypothesis of a "stochastic" origin of sporadic prion disease increasing with age[20]. Furthermore, the comparable or even earlier onset of sFI cases in comparison to FFI subjects indicate that age at onset in FFI, and possibly other genetic prion diseases, mainly depends on the specific prion strain (i.e. M2T) rather than on the presence of the *PRNP* mutation, as commonly believed.

The disease duration (mean 30 months) in our series largely overlapped with that previously reported in sFI (mean 26 months) and was, therefore, significantly longer than that of FFI carrying MM at codon 129 (about 10 months)[6-9,11-23,44-46]. Notably, one of the patients reported here had one of the longest clinical courses reported to date in sporadic human prion disease (Patient #11, 96 months)[44]. Regarding clinical manifestations, a significant and novel finding was the presence of psychiatric symptoms and a cognitive impairment, involving mainly memory, spatial and temporal orientation, language, executive functions and attention in all patients during the early or middle disease stages, which is more consistent with CJD than FFI[20]. Indeed, according to previous reports, FFI patients, especially those MM homozygous at codon 129, develop early progressive impairment of attention, vigilance and memory, resembling a progressive confusional state, but general intelligence and symbolic functions remain substantially intact until late in the disease course[7,45,47].

Besides cognitive impairment, the clinical sFI phenotype invariably comprises oculomotor and gait disturbances, dysarthria, myoclonus, extrapyramidal, cerebellar, and pyramidal signs[7,20]. Among them, the most distinctive are the oculomotor dysfunction leading to early diplopia and the postural instability with latero/retropulsion and gait impairment. The latter is like that previously reported in FFI

heterozygotes at codon 129 and is thought to be related to thalamic degeneration leading, directly or indirectly, to frontal cortical dysfunction[46].

Previously, the peculiar sleep and autonomic disturbances described in FFI were referred as "agrypnia excitata" syndrome, whose distinctive features are severe loss of sleep and persistent motor and sympathetic overactivity[7,48,49]. In our patient population of sFI, insomnia was an early and prominent manifestation in 10 cases, whereas sympathetic and motor overactivity was less common. However, as previously remarked in FI[20], in the absence of a spontaneous subjective complaint, sleep and autonomic symptoms may go unnoticed unless an accurate clinical history, video-polysomnographic and autonomic evaluation are obtained. This was particularly evident in patient #2, in whom the sleep disturbances were discovered at neurophysiological evaluation despite this not being reported by the patient. Indeed, in virtually all patients in whom video-polysomnography was performed, we found a severe reduction of total sleep time and/or completely disorganized sleep with absence of the physiological sleep figures, which characterize non-REM sleep stages N2 and N3 (spindles, K-complexes and delta waves)[7,45,48]. Moreover, in two cases we documented episodes of oneiric stupor, a behavioural state observed in agrypnia excitata, characterized by the recurrence of stereotyped and repetitive gestures mimicking the content of dreams (often referring to ordinary daily-life activities), and typically arising from subwakefulness (N1 stage)[48,49].

The present study provides, for the first time, the results of neurophysiological and biochemical evaluations of autonomic function in sFI patients. Multiple tests revealed the presence of sympathetic hyperactivity in only one of the cases with clinically reported autonomic signs. Although this discrepancy may also be attributed to the relative low sensitivity of the tests performed, or perhaps, to interlaboratory variability, taken together, our findings indicate that the clinical phenotype in patients with sFI better match that of the FFI heterozygotes MV at *PRNP* codon 129, which notoriously manifest less prominent sleep and autonomic disturbances than the MM homozygotes[7,45,46].

The diagnosis of sFI may be very difficult because of the low sensitivity of currently established investigations on which the criteria for probable sCJD are based[35]. Indeed, EEG, brain MRI and CSF 14-3-3 were all un-revealing in our cohort. Specifically, 14-3-3 was negative in all of our cases, consistent with previous reports[6,9,11,13,15,17,18,20,22]. Moreover, only two patients had EEG PSWC or MRI cortical hyperintensities suggestive of a diagnosis of probable CJD. Previously, DWI/DTI cortical hyperintensities were documented only in mixed MM2T+2C cases[13,19,23]. Prion RT-QuIC showed only 60% sensitivity for the MM2T subtype, which was similar to that reported in other sCJD subtypes linked to abnormal prion protein PrP^{Sc} type 2 and the MM codon 129 genotype[36,38]. In contrast, in all cases who underwent CSF NfL analysis, we documented high values of this biomarker[37]. Therefore, we strongly recommend the use of both prion RT-QuIC and CSF NfL based on their high specificity and sensitivity, respectively.

Furthermore, 71.4% subjects who underwent a brain FDG-PET study showed a significant bilateral hypometabolism in the thalamus in association with variable cortical involvement. These findings confirm the usefulness of FDG-PET in the diagnosis of the FI phenotype as previously reported[8,13,20,50].

In all definite cases, neuropathological assessment revealed moderate to severe neuronal loss of the anterior medial thalamus and inferior olives with a variable degree of cortical spongiform change reflecting disease duration, as previously described[6]. Other typical features of this subtype were the absence of spongiform change in the cerebellum, despite the significant gliosis and torpedo formation, and the relative sparing of striatal nuclei (i.e. caudate and putamen). The patient with the shortest duration, case 2, showed only moderate neuronal loss in the thalamus and in the olives, while in one of the cases with the longest duration (i.e. case 7) immunostaining revealed a plaque-like deposition in the cerebral cortex, cerebellum and striatum, as previously reported[12,14,21,22]. This difference is consistent with the evidence from FFI brains that the amount of prion protein deposition in the cortex and the degree of cortical pathology strongly depends on disease duration[42,43].

In conclusion, we contributed largest study to date on the clinico-pathological features and results of diagnostic investigations in sFI and the first reporting the results of the epidemiologic surveillance of sFI in Europe. We have shown that, at variance with FFI homozygotes, in sFI patients insomnia and other sleep abnormalities are usually accompanied by cognitive, psychiatric, and motor disturbances. Furthermore, we have defined the neuropsychological profile of sFI for the first time, and demonstrated a low prevalence of autonomic and neurohormonal alterations, as confirmed by multiple diagnostic investigations. The absence of pathognomonic clinical signs, the young age at onset and the relatively low sensitivity of classical diagnostic investigations (EEG, MRI, CSF 14-3-3 and t-tau) makes the clinical diagnosis of sFI challenging. However, our results indicate that video-polysomnography, brain FDG-PET, and especially CSF prion RT-QuIC and CSF NfL are the most promising diagnostic tests *in vivo*.

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Author Contributions

Conception and design of the study (SAR and PP), acquisition and analysis of the data (SAR, VR, SB, GM, DLR, GD, JHV, OW, MR, SC, AB, DI, SS, AM, PC, EG, RGW, IZ, GG, PP), and drafting the manuscript and figures (SAR, SB and PP).

Potential Conflict of Interests

Nothing to report.

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FIGURE LEGENDS

FIGURE 1. Clinical course of sFI. The mean time of appearance (in months) from disease onset is indicated for each group of symptoms/signs. *In a minority of clinical reports, the description of late symptoms was scanty or lacking; as a consequence, the numbers of patients indicated may not reliably represent the real frequency of these symptoms.

FIGURE 2. Results of serial polysomnographies. The figure shows the results of two serial polysomnographic recording performed in patient #1. (A) First polysomnography (12 months after onset): on the top, the hypnogram discloses a severe sleep loss with alteration of its macrostructure, but persistence of N2 and N3 sleep stages and of some brief REM (R) sleep episodes; on the bottom, representative epochs of N2 (see K-complex on electroencephalographic traces), N3 (see slow wave activity) and R sleep stages. (B) Follow-up polysomnography (16 months after onset): on the top, the hypnogram shows a complete loss of physiologic sleep cycles and a continuous oscillation between wakefulness (W) and subwakefulness (SW=N1 sleep stage) intermingled with brief episodes of REM sleep; on the bottom representative epochs of W, SW/N1 and R sleep stages (R.EOG and L.EOG: right and left electrooculogram; chin: superficial electromyography of mylohyoid muscle).

FIGURE 3. Neuroimaging findings. In patient #1 Brain MRI FLAIR sequences showed bilateral cortical hyperintensities, especially in the fronto-temporal lobes (A). In patient #1 (B) and #13 (C) FDG-PET showed a virtually total absence of metabolic activity within the thalamus and a variable hypometabolism of cortical regions.

FIGURE 4. Neuropathological findings and PrP^{Sc} typing. Severe neuronal loss and reactive gliosis in absence of significant spongiform change in medial-dorsal thalamic nucleus (A) and inferior olive (C). Normal controls (B and D, respectively) are shown for comparison. (E) Mild, patchy spongiform change (frontal cortex, patient #8). (F) Moderate spongiform change (temporal cortex, patient #8). (G) Status spongiosus and cortical atrophy (frontal cortex, patient #7). (H) Mild depletion of Purkinje cells, moderate Bergmann gliosis and torpedo formation (cerebellum, patient #1). (I) Absence of significant neuropathologic changes in the striatum (H&E, patient #8). (J) Prominent spongiform change in the striatum in the patient with the longest disease duration (patient #12). (K) Fine, synaptic/granular pattern of PrP^{Sc} deposition in the superficial cortical layers (PrP IHC); the box on the upper corner shows a detail at higher magnification). (L) small plaque-like PrP^{Sc} deposits in the deep cortical layers (PrP IHC). (M) Western blot analysis of PK-resistant PrP^{Sc} showing a type 2 pattern in all cases. One sCJDMM1 and one FFI are shown as controls. Type 2 was also detected in the three patients not shown in the figure (case #4: data not shown; cases #5 and 6, see Moda et al. 2012).

Table 1. Detailed demographic and clinical features of sFI cases.

Patient	Age at onset (years)	Sex	Disease duration (months)	Psychiatric	Sleep disorders *	Oculomotor / visual ¥	Postural and gait disorder	Cognitive impairment	Autonomic α	Cerebellar	Pyramidal	Extrapyramidal β	24h - motor overactivity	Dysarthria	Myoclonus	Weight loss
1	43	F	24	+	+	+	+	+	+	+	+	+	+	+	+	+
2	55	M	8	+	-	+	+	+	-	+	+	+	-	+	+	-
3	27	F	36	+	+	+	+	+	+	+	+	+	+	+	+	+
4	47	F	12	+	+	+	+	+	-	+	+	+	+	+	+	-
5	24	F	28	+	+	+	+	+	-	+	+	+	+	+	+	+
6	32	F	26	+	+	+	+	+	+	+	+	+	-	+	-	+
7	39	F	54	+	-	+	+	+	-	-	-	+	+	+	+	-
8	49	F	23	+	+	+	+	+	-	+	+	+	-	+	-	-
9	52	M	23	+	+	-	+	+	+	-	+	+	-	+	+	-
10	38	M	19	+	+	+	+	+	-	-	-	+	+	-	+	-
11	25	M	96	+	+	+	+	+	-	+	-	+	+	+	-	-
12	80	M	7	+	+	-	+	+	-	+	+	+	-	+	+	-
13	47	M	39 (still alive)	+	+	+	+	+	+	+	+	+	+	+	+	+

The grey background **i**ndicates symptoms in the first quartile of the disease course for each patient

+: reported symptom; -: symptom not reported

*: at least one among: insomnia, diurnal drowsiness, sleep vocalizations, simple or complex movements during sleep; ¥: at least one among: oculomotor dysfunction or gaze palsy associated or not

with diplopia; [α]: at least one among: systemic hypertension (often drug-resistant), tachycardia, slight pyrexia, hyperhidrosis, neurogenic urinary alterations or constipation; [β]: at least one among: hypokinetic-rigid syndrome, hyperkinetic involuntary movements (mainly chorea) or limbs dystonia

Table 2. Results of CSF biomarker assays.

N°	Time from onset (months)	14-3-3	t-tau	NfL	Prion RT-QuiC
1	11	Negative	630	12100	Positive
2	7	Negative	2350	-	-
3	12 16	Negative Negative	- 102	- 7959	- Negative
4	6	Negative	126	-	-
5	13 16 20	Negative Negative Negative	170 148 150	- - -	- - -
6	9	Negative	167	-	-
7	12	Negative	-	-	-
8	12 16	Negative Negative	- -	- -	- -
9	16	Negative	-	-	Negative
11	7	Negative	163	-	Positive
12	24 NA NA NA	Negative Negative Negative Negative	317 167 188 134	- - - -	-- - - -
13	11	Negative	226	-	-

	15	Negative	275	6695	Positive

-: not performed; NA: not available

Table 3. Results of autonomic function tests and neurohormonal assays.

Patient	Clinically reported autonomic signs	Time from onset (months)	Results of autonomic function and neurohormonal tests[α]
1	Yes	11	Tachycardia and exaggerated overshoot (60 mmHg) at Valsalva manoeuvre (standardized cardiovascular reflex tests*); normal 24 h-BcT°, BP and HR circadian rhythms; slight increase in 24h-urinary noradrenaline and dopamine.
		17	Systolic-diastolic hypertension and non-dipper pattern (24 h-BP and HR circadian rhythms); normal 24 h-BcT° circadian rhythm; slight increase in serum cortisol.
		23	Cardiovascular sympathetic hyperactivity (standardized cardiovascular reflex tests*); normal BcT° rhythm but with increased values (daytime mean: 38.1°C, nighttime mean: 36.7°C) (24 h-BcT° circadian rhythm); slight increase in blood cortisol and 24h urinary cortisol and adrenaline.
3	Yes	12	Normal values (SSR and blood cortisol, ACTH, PRL, FSH and LH levels)
6	Yes	10	Normal values (standardized cardiovascular reflex tests*)
8	No	12	Normal values (R-R interval variability; SSR)
9	Yes	9	Persistently raised systolic BP values (24 h-ambulatory BP monitoring)
13	Yes	15	Normal values (standardized cardiovascular reflex tests*)
		17	Normal values (blood cortisol, ACTH, PRL, FSH, LH, noradrenaline

			and adrenaline levels)

BcT°: body core temperature; BP: blood pressure; HR: heart rate; SSR: sympathetic skin response; ᵅ: performed tests are reported in brackets; *: head-up tilt test, Valsalva manoeuvre, deep breathing, hand grip and cold face

Table 4. Neuropathologic findings in the 12 definite cases.

Case		12	2	4	10	8	9	1	6	5	3	7	11
Duration (months)		7	8	12	19	23	23	24	26	28	36	54	96
Frontal Ctx.	SP	-*	-/+	+	++	-/+	++	++	++	+/++	++/SS(F)	SS	SS
	GL	+	+	+	++	+	++	++	++	++	++/+++	+++	+++
	NL	-	-	+	+/++	+	++	+/++	++	++	++	++	+++
Temporal Ctx.	SP	-*	-/+	-/+	++	++	++	++	++	++	++/SS(F)	++/SS	SS
	GL	+	+	+	++/+++	++	++	++	++	++	++	++/+++	+++
	NL	+	-/+	-/+	++	+/++	++	++	++	++	++	++	++
Occipital Ctx.	SP	-	+/++	-	+	-/+	+	+			+	SS	SS
	GL	+	+/++	-	++	+	+	+	NA	NA	++	+++	+++
	NL	-/+	+/++	-	+	-/+	+	+			+	++	++
Striatum	SP	-	-	-	+	-	-/+	-/+	-/+	-/+	+	+/++	++
	GL	-	-/+	-/+	+	-/+	+	+	+	+	+	+	+/++
	NL	-	-	-	+	-	-/+	-/+	-/+	-/+	+	+	+
Hippocampus (CA)	SP	-	-	-	-	-	-	-	-	-	-	-	-
	GL	-	-	-	-	-	-	-	-	-	+	+	+
	NL	-	-	-	-	-	-	-	-	-	-	-	-
Thalamus^	SP	-	-	-	-	-	-	-	-	-	-	-	-
	GL	++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	NL	++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Hypothalamus§	SP	NA	-	NA	-	-	-	-	NA	-	-	NA	NA
	GL		+		+/++	+	+/++	++		+/++	++/+++		
Inferior Olives	SP	-	-	-		-		-				-	-
	GL	++/+++	++/+++	+++	NA	++/+++	NA	+++	NA	NA	NA	+++	+++
	NL	++/+++	++/+++	++		++/+++		++/+++				+++	+++
Cerebellum	SP	-	-	-	-	-	-	-	-	-	-	-	-
	GL**	+/++	+/++	+/++	++	+/++	+/++	+/++	++	+/++	++	++	++
	NL	+	+	+	+	+	+	+	+	+	++	++	++

+: mild, ++moderate, +++severe changes; -: absent; *: a few vacuoles limited to the superficial cortical layers; ^: Dorsomedial nucleus; §: at various levels along the extension of paraventricular nucleus; neuronal loss was not assessed due to the heterogeneity of the available anatomical level among cases and the complexity of the neuronal hypothalamic cytoarchitecture **: in the molecular layer

(Bergmann's astrogliosis); CA: Hammon's horn; SP: spongiform change; GL: astrogliosis; NL: neuronal loss; SS: status spongiosus; NA= not available. F: focal changes.